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La vie est donc un feu pour la calorimétrie: half a century of calorimetry — Ingemar Wadsö at 70

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Abstract

Professor Ingemar Wadsö from the Division of Thermochemistry of the Chemical Center at the historical University of Lund in Sweden is the most important calorimetrist of the second half of the 20th century. In the way that Professor Edouard Calvet made Marseille a Mecca for calorimetrists in the middle of the last century, so Ingemar Wadsö did the same for Lund in its last part. In more than 200 publications encompassing nearly all fields of natural sciences — from simple chemical reactions to complex animal tissues and organelles — he promoted thermoanalytical investigations and their rigorous interpretation while contributing to quantitative as well as to analytical calorimetry. With about 35% of his papers in medicine and 25% in chemistry, the accents are clearly set. But it is the 17% of his publications concerning instrumental development and sophistication that made the name of I. Wadsö best known. His ampoule, batch, perfusion, titration and flow, heat conduction, microcalorimeters manufactured in Sweden by LKB Produkter AB and then by Thermometric AB, the company he founded with Jaak Suurkuusk, are found all over the world and established his most eminent contribution to this field of science.

The present paper on the occasion of Ingemar Wadsö's 70th anniversary sheds light on the uniquely important biological sector of his scientific output and indicates our deep acknowledgment of the multitude of stimuli the calorimetric family received from him in the last 40 years. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Professor Ingemar Wadsö is from the classical school of thermochemistry in which not only were the principles of the necessary physical chemistry, and especially equilibrium thermodynamics, completely understood but so were the design, construction and testing of the calorimeters used in the studies. Thus, in the 1950s, he learned from and researched with such thermochemical giants as Sunner [1], Sturtevant [2] and Skinner [3]. At the same time, during an early stage in his development, he was fully immersed at his University base in Lund with the process of building customised calorimeters [4], paying particular attention to titration [5] and precision [6] instruments. It was normal in those days for thermochemists to design their own instruments for construction in the departmental workshops and, for these projects, Ingemar's love of technical drawing proved a particular asset.

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Ingemar Wadsö is an almost unbearably young 70-years of age on 13 April, 2000. This piece is an appreciation of his unique contribution to the knowledge of living systems through the study of their heat production rate under both normal and altered physiological conditions. That he and, more especially others, were able to make such measurements was due to his genius in designing user-friendly, highly sensitive microcalorimeters.

This skill found expression in the evolution of a modular series of differential twin heat conduction micro-watt calorimeters in the middle sixties, both batch [7] and flow [8] types. Together with the earlier precision calorimeter, the microcalorimeter with a revolving module for batch experiments and a combined flow-through or mixed flow module were commercialised by the Rolls Royce of scientific instrument makers, LKB Produkter AB of Bromma, Sweden. The one regrettable change was to substitute the thermostated water bath with an air bath; but the quality of manufacture was such that, in common with many others of the LKB instrument types, dozens of the type 10700 calorimeters are still in use to this day. This marque was the cornerstone of an extensive line of work concerning instrument construction that was trend setting and gave the name for a new calorimetric generation, the *micro* reaction calorimeter.

While continuing to publish a string of good papers in classical thermochemistry (see for instance, [9] on *N,N,N*-triacetylammonia), Wadsö was quick to realise the potential of calorimetry as an analytical tool in biochemistry and related areas [10] that included microbiology [11], likening the technique to that of a spectrophotometer which can handle opaque material [12]. His long held views on the application of calorimetry to biology are best summarised in his own words in 1988: "The fact that calorimetric methods are so general makes them also very unspecific. This is a serious limitation for their practical use in many types of analytical problems. However, in biochemistry and biology the inherent specificity of the reaction systems themselves often allows the use of an unspecific analytical method. One may also note that, in particular for very complex systems, it is sometimes advantageous to use an unspecific method rather than a very specific analytical method since it is then more likely that unknown phenomena will be discovered. It is, therefore, judged that calorimetry will prove to be more important for practical analytical work on this level (cellular materials, the authors) than on the chemical level. Recent microcalorimetric studies on bacteria, blood cells and tissues certainly point in the direction of interesting new analytical determinations" [13]. This perception was reinforced as recently as 1999: "There exists hardly any measurement technique used on cellular systems which has as high resolution as calorimetry" [14], but to it this caution was added: "However, the non-specific nature of heat measurements often makes it difficult to interpret calorimetric results from complex reaction systems in sufficient detail".

The present paper is focussed on the opaque material, the 'mud and blood' Wadsö talked about at many conferences, and wrote about in the large number of papers and reviews that are the sign of a remarkably energetic man. He has never been tired of advocating the important role that measuring the heat flow rate of cells and organisms should have in understanding their physiology. In 1975 [15], the extensive list began with small animals, plant materials and microorganisms (with acknowledgement to the pioneering work of Prat in the 1960s-see [16,17]), together with striated muscle [18]. The catalogue continued by embracing the systematic studies made of the 'life processes of simple organisms like bacteria, yeast, algae and blood cells' [15]. In a further piece of advocacy 20 years later [19], he added to the list animal cells grown in culture. This was the successful result of what he described to one of us (RBK) some 15 years earlier as 'a last attempt to understanding animal cells and find money to fund research into their physiology'.

Wadsö's entry to biological studies came through two publications: 'Flow microcalorimetry as an analytical tool in biochemistry and related areas' [10] and 'Flow microcalorimetry as an analytical tool in microbiology' [11]. These introduced flow calorimetry to the life sciences and connected it forever with the name of Ingemar Wadsö. This is because, previously, skilled flow calorimetry was only used in inorganic and applied chemistry by Swietoslawski [20] with an instrument home-built before the Second World War and by Picker [21] with an eponymous commercial instrument which disappeared from the market in later years.

The prime advantage of heat conduction calorimetry is that it measures the heat flow rate (Watt) [22].

In parenthesis, it should be stated that Wadsö himself has long advocated the use of the word 'power' (P) and, more lately, thermal power because that is the unit of measurement. Indeed, this was a CODATA recommendation 20 years ago. Whatever the respective merits of the various terms, the name 'rate' conveys the meaning that the instrument gives kinetic information as well as thermodynamic data about the change in enthalpy (ΔH). The source of the heat from living matter is, of course, all the many reactions that take place in metabolism. Therefore, measuring the *rate* that heat flows from living material is a direct, quantitative assessment of its overall metabolic activity.

In advocating the potential of the calorimetric approach, Ingemar was always keen to stress to the 'mud and blood' brigade the importance of calibration that was well known to the professional calorimetrist. Since the very beginning, all the Wadsö instruments have been equipped with the same specified calibration heater of known resistance (Ω=50 ohm) to give heat by the Joule effect. However, because he designed and built calorimeters, he realised that, no matter how carefully the heater was positioned geometrically, there might well be systematic errors owing to the vessel contours and/or stirring and/or the flow of the material in solution/suspension. Therefore, he strongly advocated the use of chemical calibration using a suitable chemical reaction with a carefully measured enthalpy change.

In the present paper we will follow a schedule on Biochemical Calorimetry (Table 1) published by Wadsö in 1969 [23] and concentrate on the biological aspects of his oevre that are most familiar to us and, more crucially, seem to be the most important.

2. Review papers

Ingemar Wadsö was often asked to write review articles about special aspects of microcalorimetry or to contribute to handbooks of thermodynamics and calorimetry. He was eager to do so because he felt these are appropriate vehicles to disseminate the 'news' of this important analytical tool. The reviews comprise such instrumental topics as 'Thermometric Calorimeters' [24] in Volume 1 of the recent 'Handbook of Thermal Analysis and Calorimetry' by M.E. Brown, or the earlier classical papers 'Microcalorimeters' [25], 'Calorimetry as an Analytical Tool in Biochemistry and Biology' [15] and 'A system of micro-calorimeters and its use in biochemistry and biology' [26]. There have been many others in this mode, too numerous to identify individually but now cited as [10,12,13,27–32], as well as several introductions to the application of calorimetry in the medical and life sciences [13,19,33–37]. In many of these articles he discussed the problems which appear, that are due to the non-specific signal of the calorimeter and the necessity to make truly simultaneous measurements — at best in the same calorimetric vessel — with other biophysical sensors like pH — or oxygen electrodes and light guides, e.g. [38].

3. Instrumentation

Although Ingemar Wadsö produced a real palette of different calorimeter types and vessels, it was emphasised in Section 1 that his flow-through system [8,10,11] was for a long time the most attractive version for biologists, especially for those working

Table 1
The field of biochemical calorimetry (adapted from Wadsö [23])

O bjects	Examples	State of the system	Obtained data
Biological objects	Organelles, tissues microbes, plants, animals	III-defined systems non-equilibrium	Mainly analytical values some global data
Isolated biochemical compounds			
High molecular compounds	Proteins, nucleic acids	Purity? Structure?	(Sometimes speculative) thermodynamic data
Low molecular compounds	Peptides, aminoacids, sugars	Well-defined systems	Thermodynamic data
Model compounds (non-biochemical compounds)	Simple organic compounds	Well-defined systems	Thermodynamic data

with microorganisms [39]. Only recently the Thermometric Thermal Activity Monitor (TAM) with its effective stirring and with micro-electrodes directly in the calorimetric vessel has opened a true competition in investigations of cellular systems.

The great advantage of the flow calorimeter is the separation of the calorimeter from the fermentor in which the microbial culture is developing [40–42] or from the bioreactor in which the animal cells are growing [43,44], even for the indefinite periods under chemostat conditions. The separation (ex situ location) avoids the transfer of thermal disturbances from the culture vessel to the calorimeter that may appear in handling the biological system by stirring, aeration, sampling, addition of anti-foam agents or adjusting the pH value. These types of interference presented significant problems in the earlier calorimetric batch experiments or made them even impossible when high oxygen saturation was necessary, for instance, to achieve sufficient microbial growth. Pitfalls appeared by changed or unexpected metabolic pathways that became prominent under inappropriate environmental conditions.

The advantage of the separation of the fermentor and the calorimeter can result in a severe drawback at the same time, particularly for microbial cultures. At the usual flow rates of less than 1 $cm^3 min^{-1}$, the transport of a volume element from the culture to the calorimetric vessel takes some minutes during which the metabolic conditions — mainly the dissolved oxygen (DO) concentration — may change significantly at higher cell titres. Thus, experimental artefacts are pre-programmed that can lead to misinterpretations if no control experiments are run in parallel [45]. By modifications to a standard LKB 2107 microcalorimeter, Leiseifer [42,46] was able to increase the flow rate about 10-fold, up to 9 $cm^3 min^{-1}$, without losing sensitivity and accuracy. In this way, the transit time was reduced to about 10 s. Under such highly sophisticated, extreme conditions any changes in microbial metabolism are avoided and true kinetic investigations can be performed.

After the great success of his first type of flow-through calorimeter, Wadsö has worked with his former student and long-time colleague, Jaak Suurkuusk on a new commercial system for the last two decades [47]. This was born commercially as the LKB BioActivity Monitor and, through a process of continual development, has come to be sold by Thermometric AB, a company formed by the two of them after the takeover of LKB, as the TAM. This modular microcalorimetric system, placed in a water bath thermostat of high precision, can hold up to four very different twin calorimeters with fixed or exchangeable vessels. The latter, for example, may be used for stirred reactions in perfusion or titration experiments. The flow mode with liquid, gas or a mixture of both is applicable as well as a pure batch mode [48]. This was designed to eliminate the restrictions so often met with *classical* batch instruments or even with flow systems. The new microcalorimeter was not only used in Wadsö's Lund environment, but also worldwide from China to South Africa. Over the years at the Chemical Center in Lund, Ingemar had gathered a loyal team of highly skilled technical assistants who were an integral part of all his projects. They are Agneta Brown, Bengt Falk (mechanical workshop), Gunilla Gränz, Sven Hägg (electronic design), Gösta Pettersson (mechanical workshop) and Eva Qvarnström; plus, of course the trusted secretary, Gerd Hornemark. These were vital to Wadsö in the continuous process of improving the TAM in the following years by the design and fabrication of more effective stirrer turbines, different electrodes (oxygen, pH) (see Fig. 1) and very recently with light guides to convert it into a spectrophotometer (Fig. 2), suitable for plant cell or microbial investigations [49–52]. More details of the different vessels will be given in the following sections.

4. Chemical calibration

Wadsö [32,37,53,54] has advocated the use of triacetin for calibrating 'slow' biological reactions since the early 1980s. At that time [53], no attempt was made to quantify the kinetics of this reaction. The following second-order polynomial expansion was proposed, however, as an approach to the real thermal kinetics of the triacetin hydrolysis [53],

$$\Phi = a - bt + ct^2 \tag{1}$$

where a, b, c are the corresponding coefficients for this thermal kinetic equation. Since then, Wadsö [37] has provided a set of compositions and measured the true heat flow rate histories of these specified solutions. The major feature of the thermal kinetic equation,

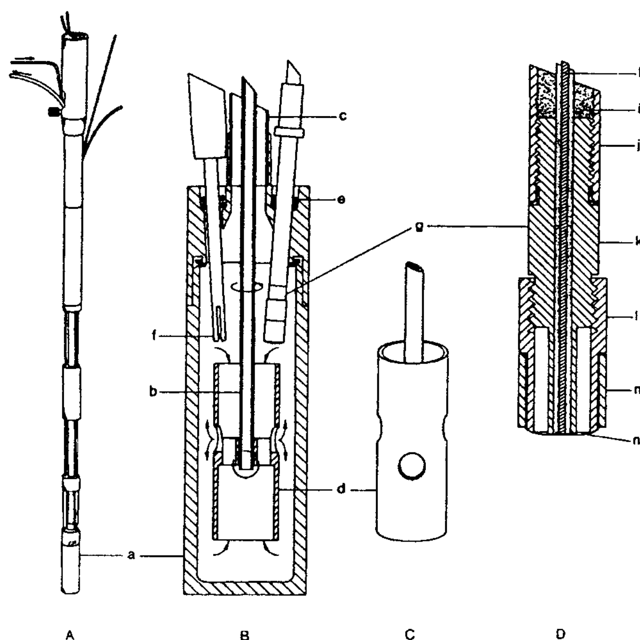


Fig. 1. (A) A combined titration/perfusion vessel equipped with a stirring device, a polarographic oxygen sensor and a combination pH-electrode (MI-414). (B) Section through the 3 cm^3 sample compartment with the electrodes mounted. The sample compartment is shown in the perfusion mode, with the turbine stirrer mounted on a hollow stirrer shaft. (C) Turbine stirrer made of Kel-F or stainless steel. (D) Section through the tip of the oxygen sensor. a — sample compartment; b — hollow stirrer shaft; c — outer steel casing; d — turbine stirrer; e — O-rings; f — combination pH-electrode with the glass membrane protected by stainless steel tube; g — polarographic oxygen sensor; h — glass-coated platinum cathode; i — epoxy resin; j — stainless steel tubing; k — silver anode; l — stainless steel membrane holder; m — Kel-F collar; n — double teflon membrane (Reproduced from [50] with permission).

Eq. (1), is that it is purely empirical, being based on a second-order polynomial expansion at a time when second virial coefficients were widely used in solution chemistry. More recently, Willson et al. [55,56] have studied the kinetics of the reaction using mathematical software (Mathcad®) and come to the conclusion that it is second order (but see [57]).

5. Microorganisms

In 1973, Boling, Blanchard and Russell published a short paper in Nature [58] about the characterisation and, conceivably, the identification of microorganism species or even strains of them by their specific calorimetric 'power–time curves'. In parenthesis, this phrase is advocated in a CODATA publication and in [59] to express the measurement of Watts as the unit of power over a period of time. Boling et al. [58] stimulated strong research activity in this direction with the hope that multichannel calorimeters could be the instruments of choice for routine microbial testing in, for example, hospitals, dairies, breweries and environmental monitoring. Different laboratories worked in this new direction, mainly with LKB flow calorimeters and in co-operation with Ingemar Wadsö [60,61]. This track was left after some years because multichannel calorimeters were not effective and the common clinical and industrial techniques by far cheaper and less difficult to handle than calorimetry. Nevertheless, this new direction was a success in emphasising the need for stronger attention to the analytical aspect of calorimetry and it led directly to the calorimetric investigation of the influences of drugs on cellular systems. This trend continues up to now with strong contributions from Lund with respect to tetracyclines, 2,4-dinitrophenol and many other compounds (see, for example, [62–65].

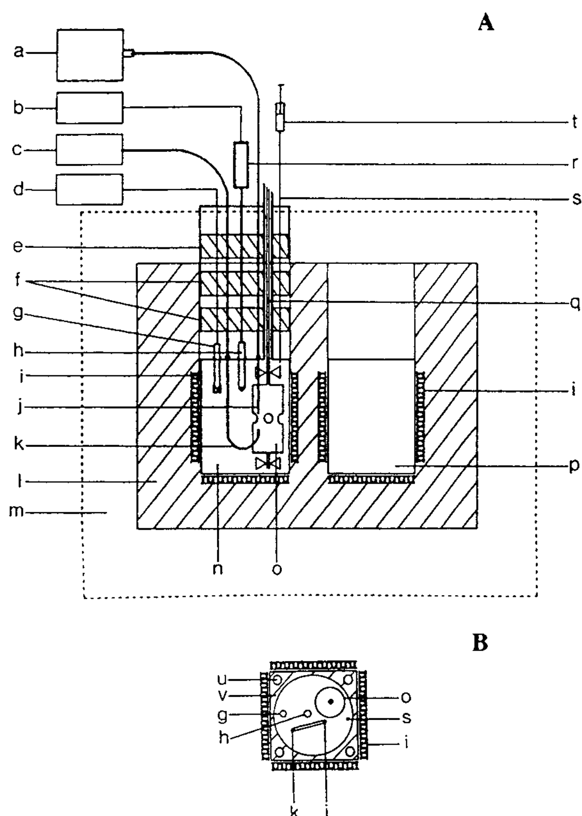


Fig. 2. (A) Schematic picture of the calorimetric assembly. (B) Horizontal section through the sample container. (a) Deuterium-halogen light source; (b) pH meter; (c) monochromator and diode array detector; (d) electronic unit of the polarographic oxygen sensor; (e) brass bolt; (f) brass bolts; (g) oxygen electrode; (h) pH electrode; (i) thermocouple plate; (j) light guide; (k) light guide; (l) heat sink; (m) thermostated water bath; (n) sample container; (o) 'turbine' stirrer; (p) reference vessel; (q) steel tube; (r) reference electrode; (s) injection needle; (t) Hamilton syringe and syringe drive; (u) hole with permanently installed calibration heater; (v) squared aluminium can (Reproduced from [52] with permission).

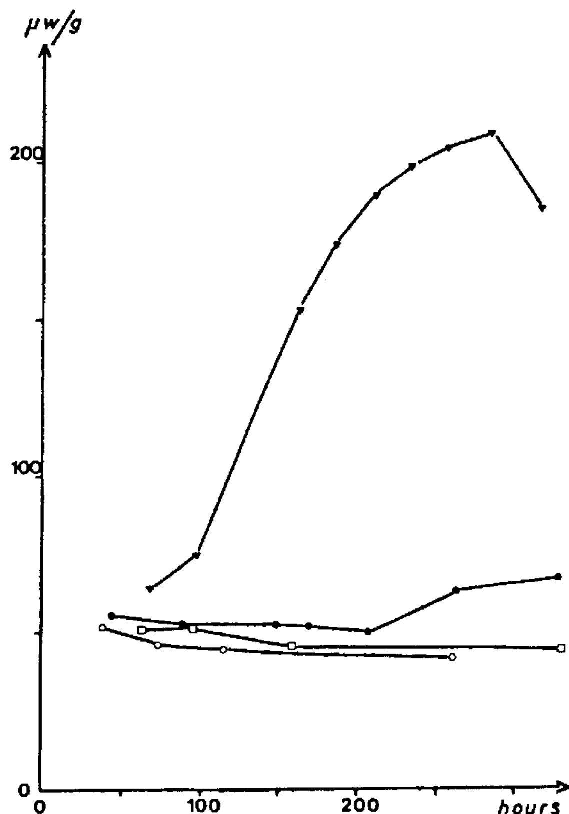


Fig. 3. Heat effects from variously fertilised soil samples. Nutrients added: cellulose powder (2%), salt mixture ($NaNO_3$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.1%; K_2HPO_4, 0.2%). The symbols are ○ untreated sample; □ salt mixture added; ● cellulose powder added; ▼ salts and cellulose added (Reproduced from [68] with permission).

6. Soil

The summarising words in an early Wadsö paper [66] on the subject of soils are: "The study of biological activity in soil involves many difficulties, owing to the great complexity of the system. *But*, ...microcalorimetry is a tool which, in combination with other methods, can be very useful for these types of studies.... . The method is also simple, rapid and precise.... . The influence of pH, moisture, temperature and various nutritional conditions can thus easily be studied".

This article was one of the first microcalorimetric papers on the bioactivity of soil. It showed the drawbacks of the method, but also the advantages of it and stimulated a line of research activities in various laboratories up to now [67]. Ingemar Wadsö participated intensively in these investigations and developed as a by-product a new ampoule technique that enabled the long-term monitoring of individual soil samples without any experimental artefact [68]. Stimulation of the microbial activity by supplementation with glucose or cellulose became the standard technique applied in many other experiments (Fig. 3). Treatment of soil specimens by heat and

irradiation together with the influence of drugs on the bioactivity were investigated as well as the effect of lowering the pH, which is of general concern because of acidification of soil and water-systems worldwide [69].

The BAM and its successor, the TAM were effectively used to study the effect of artificial irrigation, acid precipitation and liming of a spruce forest soil [70,71] and for both the moisture content and the biodegradation of organic substances in the soil [67,72]. Of course, not only instruments of the Wadsö genre were used in such investigations. To name just two *competition* papers, one is Sparling's work to assess biomass and activity in soil; it was run in a classical Calvet calorimeter with a 100 cm^3 flow vessel to enable gas exchange with the atmosphere [73]. The second is the publication of Kimura and Takahashi on heat evolution and microbial activity during glucose degradation in soil, determined with an own multiplex batch calorimeter [74].

7. Plants

Determination of heat production rates in whole plants was performed only recently because of technical difficulties, but the germination of seeds already had been monitored by *true* calorimetry (not thermometry) 50 years ago by Prat and Calvet [75,76]. This work is summarised in their famous book published in 1956 (see [77] with an English translation in 1963 [78]), the year when Ingemar Wadsö published his first, non-biological, paper [3]. The two of them established the classical power–time curve of germination after hydration of grains with three essential periods. These are the simple *physico-chemical thermogenesis*, followed by a *dead time* of nearly no heat exchange and the *biological thermogenesis* of steadily increasing rates. They then observed the influence of moisture, chemical compounds or physical factors on the curves. Even illumination and a special gas flow were given in long-term experiments of several weeks to young plants that entered their photo-state with light and would have shrunk in the dark. Other authors, for example Penon [79], continued such investigations on seeds of different origin.

Fresh stimulation for plant calorimetry and for the opening of new fields occurred in the last decade from two sides. Lee D. Hansen and his group in Provo, Utah (USA) established among other things a long-term prediction of plant growth by isothermal and temperature scanning calorimetry [80,81]. Ingemar Wadsö developed several ingenious calorimetric setups for the simultaneous determination of heat production rate and gas exchange of plant tissues in the dark [82] as well as for an effective UV and visible illumination of specimens by light guides so that true photosynthetic and photobiological experiments became possible [51]. Both approaches widen the classical static-ampoule procedure significantly and stimulate the fantasy to design experiments with these new techniques and possibilities that were unimaginable not so long ago [83]. Now calorimetric experiments that have conditions very close to the natural situation have superseded the previously artificial and therefore, questionable ones on plant tissues. Consequently, the obtained information is indeed reliable.

8. Animals

In spite of his broad spectrum of calorimetric applications and his research in animal and human tissue and organelles, no papers on whole animals have been published by Wadsö — a situation that should change significantly till his 80th anniversary! Nevertheless, the different forms of his instruments were frequently applied in studies of intact terrestrial and aquatic animals. These ranged from the protozoan *Amoeba proteus* [84], through the well-known brine shrimp *Artemia* [85,86] and an early paper on the mealworm *Tenebrio molitor* [87] to the embryos of an extremely odd mammalian, the grey short-tailed opossum, *Monodelphus domestica*, studied for questions of extrauterine development and pre-term infants [88]. A list is given as Table 2 that provides a short survey of some of the intact animals involved in calorimetric experiments with LKB and Thermometric calorimeters.

As stated earlier, Ingemar Wadsö has done a great deal of work on human and animal cells and tissues. The remaining sections of this tribute to him are concerned with this contribution to our thermal knowledge.

Table 2
Some calorimetric experiments on small animals performed by means of Wadsö calorimeters (full citation can be obtained from [89] or from the present authors)

Animal	Trivial name	Studied effects	Authors
Amoeba proteus	Amoebe, protozoa	Pinocytosis	L. Nässberger, M. Monti, 1984, 1987
Diplolaimella chitwoodi	Nematode	Ecological interactions	M.M. Pamatmat, S. Findlay, 1983
Capitella capitata	Polychaete	Ecological interactions	M.M. Pamatmat, S. Findlay, 1983
Neanthes virens	Polychaete	Ecology, toxic effects	M.M. Pamatmat, 1982
Marenzelleria viridis	Brackish water polychaete	Ecology, sulphide	A. Schneider, 1996
Hediste diversicolor	Brackish water polychaete	Ecology	J. Fritzsche, A. von Oertzen, 1995
Lumbriculus variegatus	Aquatic oligochaete	Ecology, anoxia	E. Gnaiger 1980,1983 E. Gnaiger, I. Staudigl, 1987
Mytilus edulis	Common mussel	Anoxia, physiology	E. Gnaiger, 1983 J. Widdows, A. Hawkins, 1989
Sipunculus nudus	Peanut worms	Aerobic/anaerobic metabolism	I. Hardewig et al., 1991
Planorbis corneus	Freshwater snail	Ecology, heavy metals	G.B. Joachimsohn et al., 1989
Artemia embryos	Brine shrimp	Anaerobic dormancy	S.C. Hand, E. Gnaiger, 1988
Cyclops abyssorum	Crustacean	Ecology, anoxia	E. Gnaiger, 1983
Gammarus pulex, G. tigrinus	Freshwater amphipod	Ecology, pollution (KCl)	J.H.E. Koop et al., 1995
Tenebrio molitor	Mealworm	Metabolism	G.J. Peakin, 1973
Salvelinus alpinus (eggs)	Salmonid fish	Intoxication	E. Gnaiger, 1983
Monodelphus domestica (embryos)	Grey short-tailed opossum	Extrauterine development	D. Singer, 1998

9. Human blood

It may not be realised by everyone that blood is a tissue with the cells originating in the pleuripotent, haemopoietic tissue of the bone marrow. The cells originating in the stem cells are classified as red or white, with the latter, the leucocytes, being further divided into two main categories, myeloid and lymphoid. The myeloid cells consist of three types of granulocytes, the neutrophils, the eosinophils and the basophils, together with the monocytes. The lymphoid cells are the B and T lymphocytes and the natural killer (NK) cells. The haemopoietic tissue also gives rise to the megakaryocytes that remain in the marrow and bud off many tiny cytoplasmic cell fragments, called platelets. The blood tissue is susceptible to many diseases, most dreadfully cancer, but it can also reflect the presence of disease in other tissues and cells. Besides metabolic changes in the specific cell types, the proportion of the various types alters in pathological conditions such as anaemia. Blood tissue has been studied, of course, for many years as an entity but also the various cell types can be isolated by differential centrifugation and other, more sophisticated, techniques involving antibody labelling and the use of cell sorting in flow cytometry.

Flow calorimetry is one of the obvious means to measure the metabolic activity of whole blood that in vivo normally flows in blood vessels. Most studies, however, have been performed on particular cell types separated from blood tissue and then either pumped through the flow-through vessel of the calorimeter or placed in an ampoule that is lowered into a batch calorimeter. The impetus for investigations on human blood was the possibility of detecting various clinical conditions by differences in the heat flow rate. The pioneering work was done 30 years ago in Stockholm by Klaus Levin using the Wadsö-designed LKB 10700 flow calorimeter [8] to measure the heat produced by human platelet-rich plasma, platelets (then often known as thrombocytes) and leucocytes [90]. Knowing the potential of calorimetry as an analytical tool, it was not long before Wadsö teamed up with Mario Monti in the University Hospital in Lund. Initially they undertook a series of investigations on the clinical aspects of blood tissue but later they diversified to work on other human tissues, particularly with a view to researching medical conditions and the diagnoses of them.

The first paper in the new collaboration came in 1973 [91] and involved the use of an ampoule-drop version [15,23] of the microcalorimeter to measure the basal heat production of erythrocytes isolated by

repeated centrifugation from the blood of normal human subjects. They obtained a value of 79 mW dm^{-3}, considerably lower than that found by Levin and Boyo [92], at 115 mW dm^{-3}, using flow calorimetry. This alerted them to the need to establish standardised conditions for the experiments. In 1976, Monti and Wadsö [93] confirmed that different calorimetric techniques caused dissimilar results. They also showed that the heat production rate of human erythrocytes increased linearly with the rise in pH within the physiological range. There was a 1.2% increase if the pH were raised by 0.01 unit [94]. A graduate student of Wadsö, Per Bäckman [95], later found that the pH effect was due to increases in the rates of glucose utilisation by glycolysis and the hexose monophosphate shunt. Variations in the concentration of exogenous glucose itself, in the range from 3 to 32 mmol dm^{-3}, had no effect on the heat production rate [93]. Monti and Wadsö [96] had already shown that the shunt was stimulated by methylene blue, an electron acceptor. They also showed that the heat production rate increased with the rise in temperature in the range from 32 to 42°C [94]. The temperature coefficient, Q_{10}, was 2.8, corresponding to a 10% increase in heat production at 37°C [97]. It was also in 1976 that Monti and Wadsö [93] changed to the method of isolating the erythrocytes from centrifugation to one using a gel column [98,99] and found that the heat production rate was 100 mW dm^{-3}, 25% higher than the measurement of cells after centrifugation. This was later attributed to the removal of the younger cells by the latter procedure [97].

It is typical of the rigour that the physical scientist, Wadsö, brought to biological studies that it was considered so important to regulate the conditions for obtaining the heat production rate of the erythrocytes [100]. In their first paper using the calorimeter as an analytical tool in clinical diagnosis, Monti and Wadsö [91] had found that the erythrocytes from patients with sickle cell anaemia had a higher heat production rate than those from normal subjects. They went on to show a good correlation between the individual heat production rate and the clinical condition [101]. Successful treatment of patients with the thyroid hormones, triiodothyronine and thyroxine, was found to restore heat production to normal.

In the same way that metabolic changes to erythrocytes are symptomatic of clinical conditions, so it was considered possible that altered heat production rate in the white cells could signal disease. The contribution of the various white cells and platelets to the total heat production by blood cells was shown by the group in 1975 [102]. Platelets, for instance, had a basal heat production of 60–70 fW per cell. More extensive studies on platelets isolated by a centrifugation method established the physiological conditions for the calorimetric studies [103]. The temperature coefficient was shown to be Q_{10}=2.0 at 37°C and the pH dependence was found to be six-fold less responsive — 0.02% per 0.01 pH unit — than that of erythrocytes at pH 7.4 [97]. In pursuing the possibilities of clinical diagnosis, it was shown that hypothyroid patients had a lower heat production rate (51 fW per cell) than their normal counterparts [104]. Treatment with thyroid hormone caused a restoration of a normal rate. Monti went on to make more extensive studies (reviewed in [97,99]).

One of the major goals for clinical studies is the early diagnosis of malignant blood disease. This is manifested in the various different types of white cell that grow and divide out of control instead of maturing into fully differentiated, non-growing cells. The Wadsö-Monti axis early recognised the potential importance of calorimetry in detecting the increased metabolism of rapidly growing cells. After isolating the required white cell type by a standard method, it was found using an LKB static ampoule calorimeter that the rates of heat production of lymphocytes and the tumorous lymphoid cells in Non-Hodgkin lymphoma were very markedly higher than those of cells from human subjects responding to treatment [105]. Similar studies were conducted with another type of white cell, the granulocyte, to show that those from patients with acute myelogenous leukaemia had considerably higher heat production during the acute phase of the disease than did the cells from normal subjects [106]. More details of these experiments and Monti's later independent studies on blood tissue stimulated by his early interaction with Ingemar Wadsö can be found in [97,99].

10. Muscle

In the modular design of the LKB/Thermometric BAM/TAM (see Section 3 above), Suurkuusk and

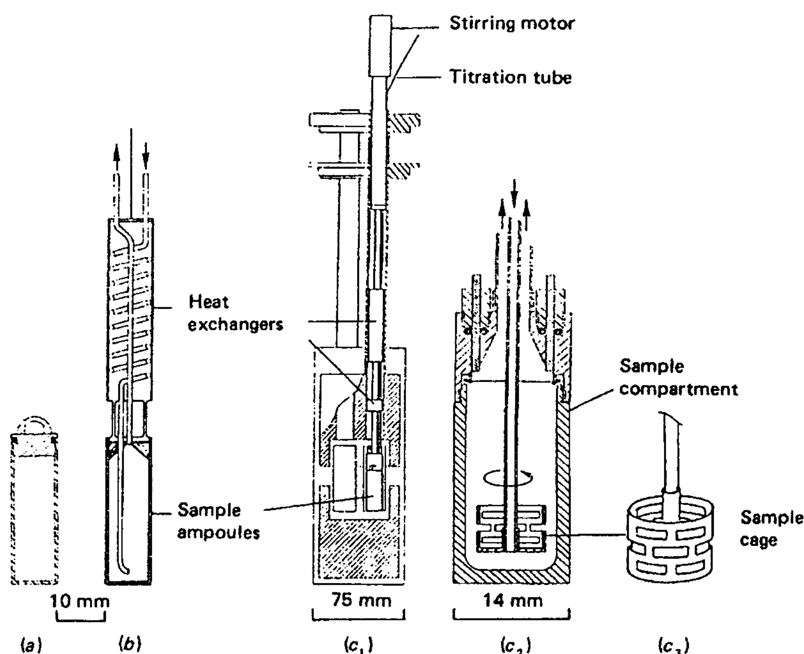


Fig. 4. Schematic representation of some parts of the TAM calorimetric equipment: (a) Simple cylindrical calorimetric vessel; (b) a type of perfusion vessel; and (c) another type of perfusion vessel with a simplified picture of the perfusion vessel inserted in the measurement position (c_1), a section through the sample compartment (c_2), and the sample cage (c_3) (Reproduced from [107] with permission).

Wadsö [47] from the start incorporated a perfusion vessel suitable for use with tissue samples [107]. In its standard form, this vessel has a stirrer but, for studies of clinical biopsies of muscle, it was replaced by a basket to hold the tissue (Fig. 4). The stirring rate was 24 rev/min^{-1} and the medium perfusion rate was 20 cm^3 h^{-1}. As an example of the type of experiment that can be done [108], human striated muscle was taken from the lateral vastus (proximal thigh) of volunteers given the β-adrenceptor blocker, propranolol and of the controls without medication. The calorimetric data showed that blockade of the sympathetic β_2-receptors decreased muscle thermogenesis by more than 50% and impaired isokinetic endurance. With his superbly proficient workshop technicians and the excellent facilities at hand, Ingemar was always able, in fact delighted, to go to the drawing board and design new vessels and adaptations to existing models. He supervised one of his postgraduate students, Per Lönnbro, in the adaptation of the TAM titration vessel so that strips of guinea-pig taenia coli smooth muscle chemically skinned with Triton X-100 could be mounted isometrically on the hooks of a modified turbine stirrer and studied for their physiological properties [109].

11. Animal and human cells from solid tissues

Much of the calorimetric data in this field has been obtained for cells adapted to grow in vitro (see Table 3), rather than for cells freshly obtained from tissues and organs (but see muscle connective tissue fibroblasts in [113]). Ingemar Wadsö was first alerted to this field as an indirect result of some work with Ljungholm on the heat production of Mollecutes [114], an area of research that would reward a revisit! The mycoplasma, as this group is commonly known, are exo-parasitic pathogens of animals and plants and a scourge of agriculture as well as animal cell culture. Thinking that calorimetry may be diagnostic of microbial infection, Wadsö, with Ljungholm primarily, looked at the infection of cells of the HeLa line by adenovirus [115]. Ingemar scribbled on a preprint he sent to one of us (RBK) at the time that ‘this paper is not very good’! Perhaps this self-criticism acted as the

Table 3
Heat flux, $J_{\Phi/N}$, for mammalian cells measured with Wadsö-designed LKB/Thermometric instruments, with ranges or standard deviations for some cell types[a]

Cell type	$J_{\Phi/N}$/pW per cell	Source
Human erythrocytes	0.01	Monti, Wadsö, 1973
Human platelets	0.06	Monti, Wadsö, 1977
Human neutrophils	2.5±0.3	Eftimiadi, Rialdi, 1982
Human lymphocytes	5	Bandmann et al., 1975
Human T-lymphoma	8±1	Schön, Wadsö, 1986a
3T3 mouse fibroblasts	17	Lönnbro, Schön, 1990
Chinese hamster ovary (CHO) 320 (recombinant)	∼23	Guan et al., 1997
KB	25	Cerretti et al., 1977
Vero	27±2	Schön, Wadsö, 1986b
Mouse lymphocyte hybridoma	30–50	Nässberger et al., 1988
HeLa-53G	31.2	McGuinness et al., 1990
Mouse macrophage hybridoma, 2C11-12	32±2	Kemp, 1992
LS-L929 fibroblasts	34±3	Hoffner et al., 1985
Chinese hamster ovary (CHO)-K1	38	Kidane et al., 1997
Rat white adipocytes	40	Nilsson-Ehle, Nordin, 1985
Human white adipocytes	49±15	Monti et al., 1980
Human melanoma, H1477	80	Nordmark et al., 1984
Hamster brown adipocytes	110	Nedergaard et al., 1979
Rat hepatocytes	329±13	Nässberger et al., 1986

[a] The tabulation is based on data in [110,111] that give the original sources — also available from the authors (modified from [112]).

spur for his concerted effort to 'understand' animal cells' (see Section 1) that was so successful in the 1980s.

Armed with some grant money and supported by some excellent research students, Wadsö in his typically thorough way made a systematic study of the conditions necessary for cultured animal cells to live in the 'foreign' environment of a calorimetric vessel (see Fig. 1). One of his mentors, Julian Sturtevant was amongst those (see review in [116]) who had described the effect of oxygen limitation when cells become too closely packed in a culture vessel [117] — the so-called 'crowding' effect. In the absence of sufficient oxygen, cells undertake substrate phosphorylation to provide the necessary adenosine triphosphate (ATP) by reducing pyruvate to lactate in glycolysis, thus increasing the production of this toxic metabolic product. There are two main ways to prevent this phenomenon. Either the cells must be grown as monolayers or they must be stirred in suspension. The former was adopted in the laboratory of one of us (IL) in that skin keratinocytes were grown on a tenterframe in a Calvet calorimeter [118]. The capacity of the Thermometric TAM is much smaller so Thorén, working with Wadsö on the heat production of rat macrophages [119], made a tiered arrangement of plates for the attachment of the cells [120]. This system was used successfully for cell toxicology studies [121]. For the most part, though, investigations have been undertaken using cells that were stirred in the perfusion vessel (see Fig. 3, vessel (c_1)) first described in 1984 [48]. In order to increase the cell density, a number of different types of bead are available that act as a substratum for the cells. One of Wadsö's research students, Arne Schön, successfully pioneered the use of stirred Cytodex beads to grow cells to high cell densities in the perfusion vessel and simultaneously to measure their thermal power [122].

In continuing their work with animal cells in the 1980s, Wadsö's group defined the physical conditions for the cells to be cultured in the perfusion vessel. Tissues and cells originating in the homeostatic conditions in tissues cannot tolerate a pH outside the range of 7.2±0.2 without deleterious effect. Besides the physical effect, it has been shown in several papers from Wadsö's laboratory (see for instance [49,123,124]) that the heat dissipated by cells is very sensitive to changes in pH and, at least for T-lymphoma cells, it peaks in the above range (see Fig. 5).

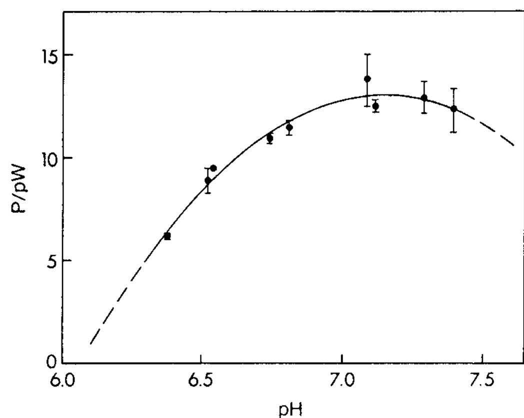


Fig. 5. The thermal power per T-lymphoma cell, P_{cell}, as a function of the medium pH (Reproduced from [123] with permission).

As one of the papers in a Collection on the occasion of Ingemar's 60th birthday [125], Bäckman [123] indicated that pH-dependency was common to many cell types but that they varied in the degree of it (in typical fashion, Ingemar thought it would be wrong for his name to appear as author of any of the papers in the Festschrift). As described in Section 9, the pH phenomenon included erythrocytes that have no mitochondria and so it was reasoned that the changes in bulk phase pH affected glycolysis [122]. Presumably, this occurs by an alteration to cytosolic pH through the Na^+, H^+ exchange in the plasma membrane. One of the reasons why lactate is so harmful to cells could be that its excretion lowers the pH of the culture medium.

The importance of maintaining cells from warm-blooded animals at 37°C was well illustrated by Bäckman [123] (see Fig. 6). The heat flux of T-lymphoma cells varied considerably between 25 and 42°C (Fig. 6a) with a pH-corrected, temperature dependence at 37°C of ~1 pW/°C (Fig. 6b). Although temperature is easily controlled in calorimetric experiments, a problem may arise in comparative studies. In this case, some of the results may be obtained under less stringent temperature control, for instance in biochemical analyses. In the case of T-lymphoma cells, Bäckman [123] recommended that the temperature control for these ex situ experiments should be within 0.1°C of the calorimetric value. He then plotted heat flux against 1/T (Fig. 6b). Based on the similarities between this curve and an Arrhenius plot, an apparent activation energy (E_a') was calculated from the slope between 25° and 37°C and found to be 87 kJ mol^{-1}. There was a rapid decline in the value of E_a' at temperatures above 37°C. Although it may not be possible to relate to the activation energy of some specific rate-limiting step, there is remarkable linearity.

The potential for the calorimetric prediction of the action of antineoplastic drugs was first realised in 1988 by Schön and Wadsö [126]. The inhibitory drug methotrexate (MTX) is very well known in genetic engineering because its target enzyme, dihydrofolate reductase (DHFR), is often incorporated into plasmids

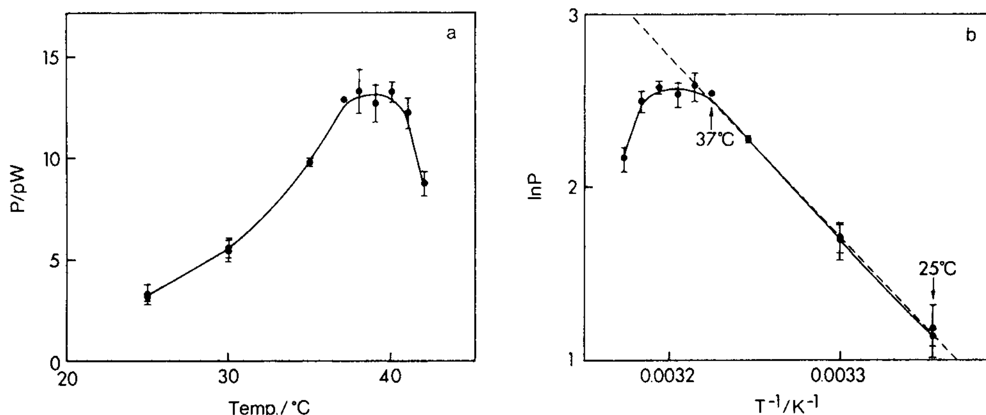


Fig. 6. (a) Changes in P_{cell}, as a function of temperature. (b) A plot of ln P_{cell} vs. $1/T$ based on the values in Fig. 6 (a). All values are corrected to pH 7.2 (Reproduced from [123] with permission).

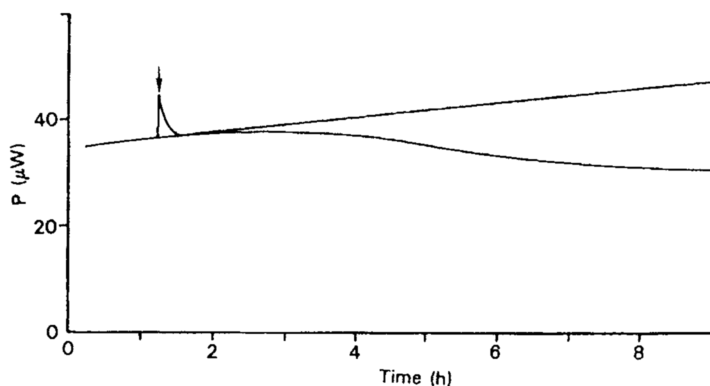


Fig. 7. Thermal power (*P*) for a sample of T-lymphoma cells where methotrexate (MTX) is injected (bottom line) and for a reference sample (top) run in parallel. The addition of MTX to a final concentration of 0.18 μM was made at the time indicated by the arrow (Reproduced from [126] with permission).

for co-amplification [116]. It effectively stops DNA synthesis by arresting de novo synthesis of purine and pyrimidines. In the experiments 12 years ago, MTX was injected into a suspension of neoplastic cells of the T-lymphoma line stirred in a Thermometric perfusion vessel by a Kel-F turbine stirrer [122]. As seen in Fig. 7, the drug had an effect on thermal power (*P*) within 2 h. A satisfactory dose–response curve was then constructed from the calorimetric data over the range of 0.02–2.00 μM MTX. This topic was examined in greater depth by members of Ingemar's research group in conjunction with some Spanish experts [127].

It will be noted that one of the changes in the Wadsö laboratory over the years has been the adoption of the perfusion vessel rather the flow vessel as the means to measure the heat produced by cells. In order to make this vessel a microanalytical 'laboratory' it was modified to include polarographic microsensors for pH and oxygen (see Fig. 1 — see [50]). It was used to monitor the metabolic activity of cells of the T-lymphoma established line (CCRF-CEM) stirred in suspension. In one experiment, there was a gaseous headspace ($0.6\ cm^3$) in the vessel so that oxygen could be recruited at the air–liquid interface. The results showed that the cells grew over approximately 30 h while there was a decrease in both the dissolved oxygen and the pH. The heat flow rate also decreased during this time. This was attributed to the decline in bulk phase pH because, as stated earlier, they had already established that this has a detrimental effect on the rate of glycolysis (see [122–124,128]).

In order to discover the longer term effects of incubating cells in medium without a gas phase for oxygen recruitment [50], the perfusion vessel was first equilibrated to 37°C with stirred medium. Then, 100 μl of concentrated cell suspension was introduced to the vessel using a Hamilton microsyringe attached to a high precision, motor-driven pump designed by Wadsö and built in his workshop [129]. The metabolic results found for the cells injected into the vessel after it had been equilibrated to 37°C for 5 h are shown in Fig. 8. With a closed vessel, a reliable estimation was made of oxygen consumption as well as thermal power (*P*) until microxic conditions prevailed at 10 h, whereupon there was a rapid decrease in the heat production (Fig. 8A). As depicted in Fig. 8B, the enthalpy balance calculations (for explanation of these sums, see [116]) revealed that oxidative processes accounted for 60% of the heat flow rate with the remainder being due to anaerobic pathways. Presumably, the main candidate would be glycolysis to produce lactate.

After 10 h, the second phase was characterised by a gradual increase in heat flow rate that was restored to its former value by 13 h (see Fig. 8A). This was entirely due to anaerobic processes (Fig. 8B). The metabolic carbon flux was found to be double the level in phase 1. This was presumably due to the much lower yield of ATP by such processes that would be dominated by the reduction of pyruvate to lactate in

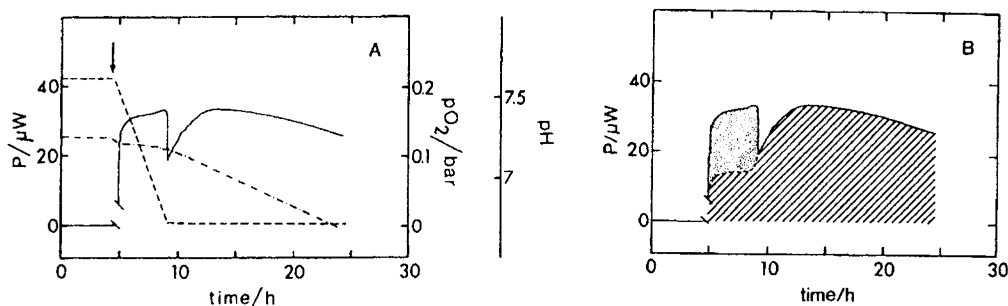


Fig. 8. (A) Parallel measurements of the heat production rate (—), oxygen activity (- - -) and pH (·–·–·) obtained from a suspension of T-lymphoma cells growing in RPMI-1640 medium. No gas phase was present during the experiment. Once the baseline had been established, the experiment was started (as indicated by the arrow) by the injection of 100 μl of concentrated cell suspension. (B) The total heat production divided into an oxygen-related part (shaded; calculated as described in the text), and a part not related to oxygen (hatched) (Reproduced from [50] with permission).

substrate phosphorylation. The increased glycolytic flux would result in a decreased pH as seen in Fig. 8A. It was not possible of course to monitor the viability of the cells over the 20 h period but one would imagine that the accumulation of toxic lactate would have a profound effect on the cells. Indirectly, this may be supposed from the fact that the heat flow rate during this phase was approximately at the same level as in phase 1, despite the fact that 15× more carbon is required to produce the same quantity of ATP as by oxidative phosphorylation.

Wadsö's interest in promoting the analytical use of calorimetry extended to clinical studies on obesity that is due to fat accumulated in white adipose tissue. White adipocytes were dissociated with collagenase from human subcutaneous adipose tissue and 'floated' on bicarbonate buffer containing glucose, insulin and albumin [130]. It came as a surprise to find that the cells from the obese patients had a lower heat production than those from the same number of normal controls (Fig. 9A). The average for the former was 26 pW per cell compared with the latter at 49 pW per cell. Adipocytes from the obese patients reflected their source and were considerably larger than those from normal people. This was emphasised by expressing the difference in terms of tissue weight (Fig. 9B). 'Obese' adipose tissue produced 40 $\mu W\ g^{-1}$ whereas the normal tissue evolved 133 $\mu W\ g^{-1}$. The presence of glucose as the catabolic substrate and of insulin was found to be necessary; in their absence the heat production rate was only 25% of the control value [131]. In a later study, measurement was made of the heat production by adipocytes from obese subjects before and after supervised weight reduction on a balanced diet and compared with lean individuals [132]. The value for the latter was confirmed at 133 $\mu W\ g^{-1}$. Before losing weight, the adipocytes obtained by gluteal biopsy from a selected group of obese patients produced heat at the rate of 40 $\mu W\ g^{-1}$. After weight loss of 13 kg (mean), the heat production rate was 65 $\mu W\ g^{-1}$. The medical part of the team then went on to conduct further research into, for instance, the effects of gastroplasty on adipocyte metabolism [133].

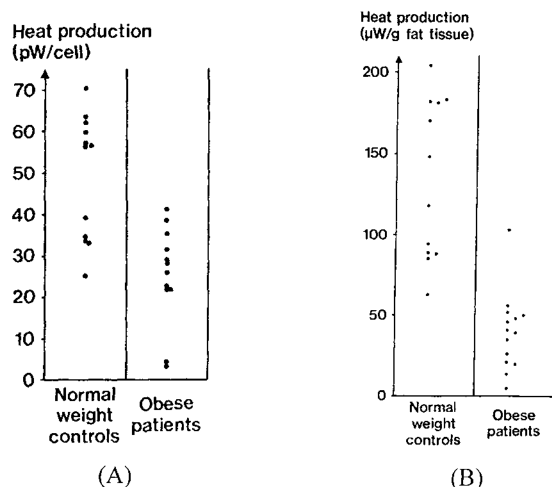


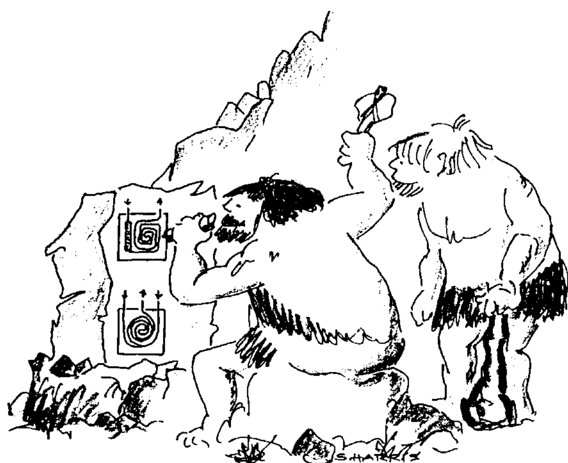
Fig. 9. The heat flow rate of adipocytes in obese patients and in lean control subjects, expressed as μW per cell (A) and as μW per gram of tissue (B). (Reproduced from [130] with permission).

12. Conclusions

This survey of Ingemar Wadsö's calorimetric activities — seen from the perspective of two biologists — is intended to show his influence on modern microcalorimetry and its renascence in the post-Calvet era, as well as his concatenation with various scientific disciplines and a manifold of research groups all over the world. At the beginning of this century his Bioactivity Monitor and his Thermal Activity Monitor have become true successors by their importance to Professor Edouard Calvet's famous calorimeter that reigned in the middle of the previous century. They gave and continue to give new impetus to calorimetry in general and to open fields that, in a stepmotherly fashion, are successors to those of earlier times — for instance plant metabolism.

Ingemar Wadsö recently wrote: "...based on the concept of combining isothermal microcalorimetry with specific analytical instruments and by a wealth of information from 30 years of method work, a new optimism can now be sensed". [19]. The authors share this opinion and acknowledge with great pleasure and admiration that a highly significant contribution to this progress and to such a hope was gifted to the scientific community by Ingemar Wadsö during more than 40 years of truly innovative research.

Pröva icke vart varje ditt steg för dig:
Endast den som ser långt hittar rätt.
Dag Hammerskjöld, Markings, 1964.



A freely interpreted, artistic image of prehistoric human artefacts with evident 10700 lineage from Southern Sweden (adapted from [134]).

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